

New technique for IDing proteins secreted by cells developed

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(Phys.org)—Researchers from North Carolina State University have developed a new technique to identify the proteins secreted by a cell. The new approach should help researchers collect precise data on cell biology, which is critical in fields ranging from zoology to cancer research.

The work is important because cells communicate by secreting proteins. Some of the proteins act on the cell itself, telling it to grow or multiply, for example. But the proteins can also interact with other cells, influencing them to perform any [biological function](#).

Traditionally, scientists who wanted to identify these proteins [cultured cells](#) and then used mass spectrometry to determine which proteins appeared in the medium the cell was grown on. This has drawbacks, because the proteins of interest are fairly rare compared to the proteins that are already in the medium – which are used to grow and support the cells in the first place. Further, any attempts to culture the cells without these background, supporting proteins affects [cell behavior](#) – skewing the sample.

The new approach takes advantage of the fact that each cell "packages" its proteins in its "secretory pathway." Each cell synthesizes the protein and passes it through this pathway, essentially placing it in a bag-like membrane before it is passed out of the cell.

In their new technique, researchers take a sample of cells and isolate the

secretory pathway organelles, which contain the proteins. The researchers then use [mass spectrometry](#) to analyze the contents of the [organelles](#), in order to see which proteins were being secreted by the cell. Using this approach, the researchers were able to identify proteins that are secreted by human [embryonic stem cells](#).

"This gives us a snapshot of exactly what a cell was secreting at that point in time," says Dr. Balaji Rao, an assistant professor of chemical and biomolecular engineering at NC State and co-author of a paper describing the work.

This new method eliminates the problems related to the proteins found in cell culture media. But it also allows researchers to track changes in the proteins released by a cell in response to a stimulus, such as exposure to a chemical. This can be done by taking samples at various points in time after cells have been exposed to the stimulus.

And, in principle, this technique would also allow researchers to identify which proteins any specific type of cell is secreting when in a mixed population of cells.

"As long as you can separate the cells you are interested in, this should be possible," says Rao. "And that is important, because most tissues are made up of heterogeneous populations of cells – and communication between those cells is biologically significant."

More information: The paper, "Targeted proteomics of the secretory pathway reveals the secretome of mouse embryonic fibroblasts and human embryonic stem cells," was published online Sept. 15 in the journal *Molecular & Cellular Proteomics*.

www.mcponline.org/content/earl...9/15/mcp.M112.020503

Abstract:

Proteins endogenously secreted by human embryonic stem cells (hESCs) and those present in hESC culture medium are critical regulators of hESC self-renewal and differentiation. Current MS-based approaches for identifying secreted proteins rely predominantly on MS analysis of cell culture supernatants. Here we show that targeted proteomics of secretory pathway organelles is a powerful alternate approach to interrogate the cellular secretome. We have developed procedures to obtain subcellular fractions from mouse embryonic fibroblasts (MEFs) and hESCs that are enriched in secretory pathway organelles, while ensuring retention of the secretory cargo. MS analysis of these fractions from hESCs cultured in MEF conditioned medium (MEF-CM) or MEFs exposed to hESC medium revealed 99 and 129 proteins putatively secreted by hESCs and MEFs, respectively. Of these, 53 and 62 proteins have been previously identified in cell culture supernatants of MEFs and hESCs respectively, thus establishing the validity of our approach. Furthermore, 76 and 37 putatively secreted proteins identified in this study, in MEFs and hESCs respectively, have not been reported in previous MS analyses. Identification of low abundance secreted proteins by MS analysis of cell culture supernatants typically necessitates the use of altered culture conditions such as serum-free medium. However, an altered medium formulation might directly influence the cellular secretome. Indeed, we observed significant differences between the abundances of several secreted proteins in subcellular fractions isolated from hESCs cultured in MEF-CM and those exposed to unconditioned hESC medium for 24 hours. By contrast, targeted proteomics of secretory pathway organelles does not require the use of customized media. We expect that our approach will be particularly valuable in two contexts highly relevant to hESC biology – to obtain a temporal snapshot of proteins secreted in response to a differentiation trigger, and to identify proteins secreted by cells that are isolated from a heterogeneous population.

Provided by North Carolina State University

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